A. Specific Aims.

I. To continue the clinical and laboratory investigation of a large series of cases of six hereditary and generalized disorders of connective tissue. Although all families do not have affected members still living, the total number of families with at least one affected member are, for each syndrome, as follows:

The Marfan syndrome	76
The Ehlers-Danlos syndrome	14
Osteogenesis imperfecta	112
Pseudoxanthoma elasticum	20
The Hurler syndrome	28
Fibrodysplasia ossificans progressiva	7

The studies on patients are of these types:

a. Genetic and statistical
b. Clinical
c. Chemical
d. Histopathologic

II. Comparative genetic pathology of connective tissue diseases:

a. The osteogenesis imperfecta - like disease of Burmese and Siamese cats.
b. Other generalized connective tissue disorders in animals if and when discovered.

B. Methods of Procedure.

Ia. The genetic and statistical studies of each syndrome will vary but in general the following aspects will be explored.

1. Survivorship. Particularly in the Marfan syndrome, this aspect will be investigated using life-table techniques and comparing affected persons with unaffected siblings.

2. The sex ratio, both for the total series and for each of the components of each syndrome; sex-difference in severity (in the Marfan syndrome, particularly) as measured by age of death.

3. The Lenz-Hogben method will be employed to evaluate the goodness of fit of observed with expected according to the dominant hypothesis, in the Marfan syndrome using the some 100 sibships on which complete data are available (many of our families have more than one sibship usable in such an analysis).

4. What proportion of cases of the Marfan syndrome arise by new mutation? In what proportion of families is there only one affected person? What proportion of new mutations are necessary to account for the deficiency of expected affected sibs in the analysis in ♀?

5. What is the relationship between so-called osteogenesis imperfecta congenita and OI tarda? Overlap families are continually being sought.

6. Total ascertainment in Baltimore and perhaps in Maryland for osteogenesis imperfecta (OI) will be attempted. It is felt that diagnostic difficulties in the other two "dominant" states -- the Marfan syndrome and the Ehlers-Danlos syndrome -- make anything approaching total ascertainment impossible.

In OI estimation of mutation rate will be attempted by both the direct and the indirect methods.

7. Total ascertainment will also be attempted for the Hurler syndrome. The existence of two genotypic varieties -- one an autosomal recessive and one a sex-linked recessive -- complicates estimation of gene frequency. However, the sex ratio in all cases will be used as the starting point for estimating what proportion of all cases are of the sex-linked recessive variety -- assuming that males and females are equally represented in the autosomal recessive group. The results will be compared with those of Lamy (J. génét. humaine 6:15, 1957) based on reports in the literature and his own series in Paris.

8. Linkage studies will be attempted in the case of the Marfan syndrome and osteogenesis imperfecta. The accumulated pedigree data will be analyzed by means of an electronic computer (IBM 704) for which linkage programming is currently being done in this department.

Ib. Clinical studies.

1. The prognosis of aortic regurgitation in the Marfan syndrome. Through survey of ectopia lentis cases and from other sources, cases of early asymptomatic aortic regurgitation in the Marfan syndrome have been ascertained. The course of these cases will be followed.

2. Joint elasticity will be studied in each of these syndromes by Dr. Richard J. Johns and Verna Wright using two methods they have developed. Especially the loose-jointedness of the Marfan syndrome, the Ehlers-Danlos syndrome and osteogenesis imperfecta and the stiff joints of the Hurler syndrome will be studied serially and compared with the findings in unaffected sibs and in other normals.

Ic. Chemical studies.

All the chemical studies will be applied to cases of all the syndromes. However, below the specific entity in which the approach is thought to be most promising will be indicated. Those procedures which will be performed in our own laboratory are indicated.

(1) In the Marfan syndrome serum mucoproteins will be determined. These have been reported (by Bacchus, Am. J. Med. Oct., 1958) to be depressed in affected members of one family. This will be done in our own laboratory.
(2) Urinary excretion of hydroxyproline. This is reportedly increased in some cases of the Marfan syndrome (Lancet 2: 994, 1958). For this aspect collaborative arrangements have already been worked out with Dr. Albert Sjoerdama, National Heart Institute.
(3) In the Ehlers-Danlos syndrome, serum will be sent to Hall and Saxl in Tunbridge's department at Leeds. They have reported increased serum elastase inhibitor in this disorder (Tunbridge: Heberden lecture (1956). Ann. Rheum. Dis., 16: 6, 1957).
(4) In the Hurler syndrome we will continue to send urine to Dr. Karl Meyer for isolation, identification and quantitation of mucopolysaccharides.
(5) In the Hurler syndrome the simplified screening test for mucopolysaccharide in urine, developed by Dorfman and Lorincz (Pediatrics 1958) and using acidified bovine albumin, will be evaluated.
(6) In the Hurler syndrome and others blood will be sent to Dr. [illegible] for serum chondroitin sulfate determination.
(7) In all these syndromes, Dr. Mary B. Stevens of the Connective Tissue Division of the Department of Medicine, expects to do serum hexosamine and other determinations.

(8) We have several patients in whom the differential diagnosis between the Hurler syndrome and Morquio's syndrome is uncertain on clinical grounds. Mucopolysaccharide studies of the urine will be performed to attempt this differentiation.

4. Histopathologic studies: As tissue becomes available, special studies are to be ~~being~~ done by Dr. Richard H. Follis in Washington and Dr. Walker of the Department of Anatomy at Hopkins.

II. Arrangements have been made to obtain Siamese or Burmese cats born with osteogenesis imperfecta. The following studies will be done:

1) Clinical

a. Does deafness develop?
b. What is the effect of puberty on the incidence of fracture?
c. What of the reports that estrogens and androgens are remarkably helpful in the feline disease?

2) Histopathologic

~~All~~ arrangements have been made with Dr. Richard H. Follis (Washington) to make histologic studies of the bones and soft tissues.

Dr. Stacey Guild (ENT Dept., J.H.U.) will do a histologic survey of the ears and a more detailed investigation if there is clinical evidence of deafness.

3) Chemical.

Dr. Karl Meyer (N.Y.C.) and Dr. Jerome Gross (Boston) have expressed interest in obtaining tissues from these animals.

4) Genetic.

OI in cats is thought to be transmitted as an autosomal recessive. It is planned to attempt confirmation by breeding affected sons with their mothers. (The occurrence of a mode of inheritance different from that in man does not necessarily indicate a difference in the basic biochemical defect.)

We are on the look-out for other hereditary disorders of connective tissue in animals.

C. Significance of this Research.

Studies of hereditary disorders of connective tissue have significance, firstly, in their own right, and secondly, in connection with the light they can shed on the biology of normal connective tissue.

D. Facilities Available.

~~This group of~~ patients in several diagnostic categories are being followed in the Moore Clinic (of the Johns Hopkins Hospital) of which the chief investigator is physician-in-charge.

A statistician part-time -- Dr. Helen B. Abbey -- will participate in the analyses involved in the study. Her salary support is derived from a Medical Genetics Graduate Training Grant.

Adequate facilities for maintaining animals are available in the Department of Medicine.

Two rooms outfitted as a chemistry laboratory are available.

Personnel

Technician (general duty)	$3600.
Fellow	4500.
Social Security	162.

Permanent equipment

~~Fume hood~~	~~1000.~~
Animal cages	500.

Consumable supplies

Animal purchase, feed, care	1500.
Stenographic supplies and services	500.
Chemicals, photographic services	1000.
Blood groupings	500.

Travel

Meetings and lab conferences	500.
Field trips for laboratory studies	500.

Other expenses

Clinic fees, expenses of in-patient hospital admission for special studies, X-rays, biopsies, etc.	1000.
	$15,262.

15%

Previous Work

See Progress Report.

Personal Publications

McKusick, V.A.: Heritable Disorders of Connective Tissue, St. Louis: C.V.Mosby, 1956 (Revised edition, 1959).

McKusick, V.A.: Genetic factors in diseases of connective tissue. A survey of the current status of knowledge. Am. J. Med., Feb., 1959.

McKusick, V.A.: Hereditary diseases of connective tissue. Bull. New York Acad. Med., March, 1959.

Results obtained by others.

Pertinent references have been given in the section on Methods of Procedure. Extensive and critical review of the literature is available in my monograph.

Biographical Sketches

Victor A. McKusick. Born in Maine, 1921. Tufts College 1940-1943. Johns Hopkins University School of Medicine 1943-1946 (M.D.). House staff training, Johns Hopkins Hospital 1946-1948 and 1950-1952. Cardiovascular research, U.S.P.H.S. Hospital, Baltimore, 1948-1950. From 1952 to present: Full time academic staff, Department of Medicine, Johns Hopkins University School of Medicine. Present positions: Associate professor of Medicine; assistant professor of epidemiology; physician, Johns Hopkins Hospital; Chief, Division of Medical Genetics, Dept. of Medicine; physician-in-charge, The Joseph Earle Moore Clinic.

Helen B. Abbey.